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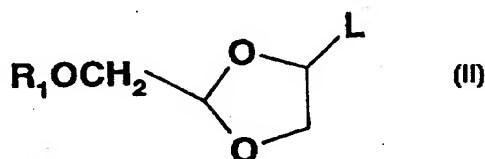
INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification ⁶ : C07D 411/04, C07H 19/06</p>	<p>A1</p>	<p>(11) International Publication Number: WO 97/21706 (43) International Publication Date: 19 June 1997 (19.06.97)</p>
<p>(21) International Application Number: PCT/CA96/00845 (22) International Filing Date: 13 December 1996 (13.12.96) (30) Priority Data: 9525606.1 14 December 1995 (14.12.95) GB (71) Applicant (for all designated States except US): BIOCHEM PHARMA INC. [CA/CA]; 275 Armand-Frappier Boulevard, Laval, Quebec H7V 4A7 (CA). (72) Inventors; and (75) Inventors/Applicants (for US only): MANSOUR, Tarek [CA/CA]; Apartment 102, 3555 University Street, Montreal, Quebec H3A 2B1 (CA). CIMPOIA, Alex [CA/CA]; Apartment 22, 3550 Ridgewood Avenue, Montreal, Quebec H3V 1C2 (CA). BEDNARSKI, Krzysztof [CA/CA]; 237 Labri Avenue, Laval, Quebec H7N 5R6 (CA). (74) Agents: O'CONNOR, Edward, B. et al.; Scott & Aylen, 60 Queen Street, Ottawa, Ontario K1P 5Y7 (CA).</p>		<p>(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</p> <p>Published With international search report.</p>

(54) Title: METHOD AND COMPOSITIONS FOR THE SYNTHESIS OF DIOXOLANE NUCLEOSIDES WITH β -CONFIGURATION

(57) Abstract

The present invention relates to methods and compositions for preparing biologically important nucleoside analogues containing 1,3-dioxolane sugar rings. In particular, this invention relates to the stereoselective synthesis of the beta (cis) isomer by glycosylating the base with an intermediate of formula (II) below a temperature of about -10 °C, wherein R₁ and L are as defined herein.



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METHOD AND COMPOSITIONS FOR THE
SYNTHESIS OF DIOXOLANE NUCLEOSIDES
WITH β -CONFIGURATION

5

FIELD OF THE INVENTION

The present invention relates to methods and compositions
for preparing nucleoside analogues containing dioxolane
10 sugar rings. In particular, the invention relates to the
stereoselective synthesis 1,3-dioxolane nucleosides having
 β or cis configuration.

15 BACKGROUND OF THE INVENTION

Nucleosides and their analogues represent an important
class of chemotherapeutic agents with antiviral,
anticancer, immunomodulatory and antibiotic activities.
20 Nucleoside analogues such as 3'-azido-3'-deoxythymidine
(AZT), 2',3'-dideoxyinosine (ddI), 2',3'-dideoxycytidine
(ddC), 3'-deoxy-2',3'-didehydrothymidine (dT) and (-)-2'-
deoxy-3'-thiacytidine (3TC™) are clinically approved for
the treatment of infections caused by the human
25 immunodeficiency viruses. 2'-Deoxy-2'-methylidenecytidine
(DMDC, Yamagami et al. *Cancer Research* 1991, 51, 2319) and
2'-deoxy-2',2'-difluorocytidine (gemcytidine, Hertel et
al. *J. Org. Chem.* 1988, 53, 2406) are nucleoside analogues
with antitumor activity. A number of C-8 substituted
30 guanosines such as 7-thia-8-oxoguanosine (Smee et al. *J.*
Biol. Response Mod. 1990, 9, 24) 8-bromoguanosine and 8-
mercaptoguanosine (Wicker et al. *Cell Immunol.* 1987, 106,
318) stimulate the immune system and induce the production
of interferon. All of the above biologically active
35 nucleosides are single enantiomers.

Recently, several members of the 3'-heterosubstituted class of 2',3'-dideoxynucleoside analogues such as 3TC™ (Coates et al. *Antimicrob. Agents Chemother.* 1992, 36, 202), (-)-FTC (Chang et al. *J. Bio. Chem.* 1992, 267, 13938-13942) (-)-dioxolane C (Kim et al. *Tetrahedron Lett.* 1992, 33, 6899) have been reported to possess potent activity against HIV and HBV replication and possess the β -L absolute configuration. (-)-Dioxolane C has been reported to possess antitumor activity (Grove et al. *Cancer Res.* 1995, 55, 3008-3011). The dideoxynucleoside analogues (-)-dOTC and (-)-dOTFC (Mansour et al. *J. Med. Chem.* 1995, 38, 1-4) were selective in activity against HIV-1.

For a stereoselective synthesis of nucleoside analogues, it is essential that the nucleobase be introduced predominately with the desired relative stereochemistry without causing anomerization in the carbohydrate portion. One approach to achieve this is to modify the carbohydrate portion of a preassembled nucleoside by a variety of deoxygenation reactions (Chu et al. *J. Org. Chem.* 1989, 54, 2217-2225; Marcuccio et al. *Nucleosides Nucleotides* 1992, 11, 1695-1701; Starrett et al. *Nucleosides Nucleotides* 1990, 9, 885-897, Bhat et al. *Nucleosides Nucleotides* 1990, 9, 1061-1065). This approach however is limited to the synthesis of those analogues whose absolute configuration resembles that of the starting nucleoside and would not be practical if lengthy procedures are required to prepare the starting nucleoside prior to deoxygenation as would be the case for β -L dideoxynucleosides. An alternative approach to achieve stereoselectivity has been reported which requires assembling the nucleoside analogue by a reaction of a base or its synthetic precursor with the carbohydrate portion under Lewis acid coupling procedures or SN-2 like conditions.

It is well known in the art that glycosylation of bases to dideoxysugars proceed in low stereoselectivity in the absence of a 2'-substituent on the carbohydrate rings capable of neighboring group participation. Okabe et al. (J. Org. Chem. 1988, 53, 4780-4786) reported the highest ratio of β : α isomers of ddC of 60:40 with ethylaluminum dichloride as the Lewis acid. However, with a phenylselenenyl substituent at the C-2 position of the carbohydrate (Chu et al. J. Org. Chem. 1980, 55, 1418-1420; Beach et al. J. Org. Chem. 1992, 57, 3887-3894) or a phenylsulfenyl moiety (Wilson et al. Tetrahedron Lett. 1990, 31, 1815-1818) the β : α ratio increases to 99:1. To overcome problems of introducing such substituents with the desired α -stereochemistry, Kawakami et al. (Nucleosides Nucleotides 1992, 11, 1673-1682) reported that disubstitution at C-2 of the sugar ring as in 2,2-diphenylthio-2,3-dideoxyribose affords nucleosides in the ratio of β : α = 80:20 when reacted with silylated bases in the presence of trimethylsilyltriflate (TMSOTf) as a catalyst. Although this strategy enabled the synthesis of the β -anomer, removal of the phenylthio group proved to be problematic.

Due to the limited generality in introducing the C-2 substituent stereoselectively, synthetic methodologies based on electrophilic addition of phenyl sulfenyl halides or N-iodosuccinimides and nucleobases to furanoid glycal intermediates have been reported (Kim et al. Tetrahedron Lett. 1992, 33, 5733-5376; Kawakami et al. Heterocycles 1993, 36, 665-669; ; Wang et al. Tetrahedron Lett. 1993, 34, 4881-4884; El-laghdach et al. Tetrahedron Lett. 1993, 34, 2821-2822). In this approach, the 2'-substituent is introduced in situ however, multistep procedures are needed for removal of such substituents.

SN-2 like coupling procedures of 1-chloro and 1-bromo 2,3-dideoxysugars have been investigated (Farina et al. *Tetrahedron Lett.* 1988, 29, 1239-1242; Kawakami et al. *Heterocycles* 1990, 31, 2041-2053). However, the highest ratio of β to α nucleosides reported is 70:30 respectively.

In situ complexation of metal salts such as SnCl_4 or $\text{Ti}(\text{O-Pr})_2\text{Cl}_2$ to the α -face of the sugar precursor when the sugar portion is an oxathiolanyl or dioxolanyl derivative produces β -pyrimidine nucleosides (Choi et al. *J. Am. Chem. Soc.* 1991, 113, 9377-9379). Despite the high ratio of β - to α -anomers obtained in this approach, a serious limitation with enantiomerically pure sugar precursor is reported leading to racemic nucleosides (Beach et al. *J. Org. Chem.* 1992, 57, 2217-2219; Humber et al. *Tetrahedron Lett.* 1992, 32, 4625-4628; Hoong et al. *J. Org. Chem.* 1992, 57, 5563-5565). In order to produce one enantiomeric form of racemic nucleosides, enzymatic and chemical resolution methods are needed. If successful, such methods would suffer from a practical disadvantage of wasting half of the prepared material.

As demonstrated in the above examples, the art lacks an efficient method to generate β -nucleosides. In particular, with sugar precursors carrying a protected hydroxymethyl group at C-4', low selectivity is encountered during synthesis of β -isomers or racemization problems occur. Specifically, the art lacks a method of producing stereoselectively dioxolanes from sugar intermediates carrying a C-2 protected hydroxymethyl moiety without racemization. Therefore, a general stereoselective synthesis of biologically active β -nucleoside analogues is an important goal.

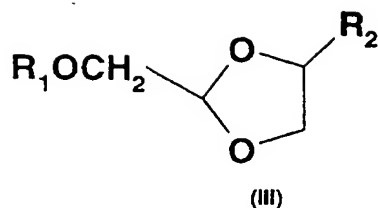
International patent application publication no. WO92/20669 discloses a method of producing dioxolanes stereoselectively by coupling sugar intermediates carrying C-2 ester moieties with silylated nucleobases and subsequently reducing the C-2 ester group to the desired hydroxymethyl group. However, over reduction problems in the pyrimidine base have been disclosed (Tse et al. *Tetrahedron Lett.* 1995, 36, 7807-7810).

10 Nucleoside analogues containing 1,3-dioxolanyl sugars as mimetics of 2',3'-dideoxyfuranosyl rings have been prepared by glycosylating silylated purine and pyrimidine bases with 1,3-dioxolanes containing a C-2 hydroxymethyl and C-4 acetoxy substituents. The crucial coupling reaction is mediated by trimethylsilyltriflate (TMSOTf) or iodotrimethylsilane (TMSI) and produces a mixture of β and α -anomers in 1:1 ratio (Kim et al. *J. Med. Chem.* 1992, 35, 1987-1995 and *J. Med. Chem.* 1993, 36, 30-37; Belleau et al. *Tetrahedron Lett.* 1992, 33, 6948-6952; and Evans et al. *Tetrahedron Asymmetry* 1992, 4, 2319-2322). By using metal salts as catalysts the β -nucleoside is favoured (Choi et al. *J. Am. Chem. Soc.* 1991, 113, 9377-9379) but racemization or loss of selectivity become a serious limitation (Jin et al. *Tetrahedron Asymmetry* 1993, 4, 2111-2114).

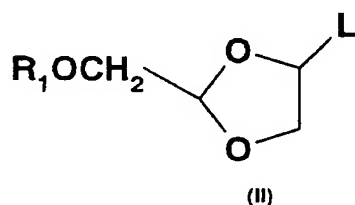
SUMMARY OF THE INVENTION

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According to an aspect of the present invention, there is provided a process for producing a β -nucleoside analogue compound of formula (III):

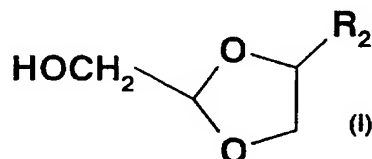


and salts thereof, wherein R_1 is a hydroxyl protecting group; and R_2 is a purine or pyrimidine base or an analogue thereof, the process comprising glycosylating said purine or pyrimidine base at a temperature below about -10°C , with an intermediate of formula (II):



10 wherein L is halogen.

Subsequent to glycosylation, the compound of formula (III) may then undergo deprotection of the hydroxyl protecting group R_1 to give a 1,3-dioxolane nucleoside analogue of formula (I)



wherein R_2 is as previously defined.

20

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a novel method for producing dioxolane nucleoside analogues by coupling sugar

precursors carrying a C-2 protected hydroxymethyl group with purine or pyrimidine nucleobases in high yield and selectivity in favour of the desired β -isomers.

- 5 A « nucleoside » is defined as any compound which consists of a purine or pyrimidine base or analogue or derivative thereof, linked to a pentose sugar.

- A « nucleoside analogue or derivative » as used
10 hereinafter is a compound containing a 1,3-dioxolane linked to a purine or pyrimidine base or analog thereof which may be modified in any of the following or combinations of the following ways: base modifications, such as addition of a substituent (e.g. 5-fluorocytosine)
15 or replacement of one group by an isosteric group (e.g. 7-deazaadenine); sugar modifications, such as substitution of hydroxyl groups by any substituent or alteration of the site of attachment of the sugar to the base (e.g. pyrimidine bases usually attached to the sugar at the N-1
20 site may be, for example, attached at the N-3 or C-6 site and purines usually attached at the N-9 site may be, for example, attached at N-7.

- A purine or pyrimidine base means a purine or pyrimidine
25 base found in naturally occurring nucleosides. An analogue thereof is a base which mimics such naturally occurring bases in that its structure (the kinds of atoms and their arrangement) is similar to the naturally occurring bases but may either possess additional or lack certain of the
30 functional properties of the naturally occurring bases. Such analogues include those derived by replacement of a CH moiety by a nitrogen atom, (e.g. 5-azapyrimidines, such as 5-azacytosine) or conversely (e.g., 7-deazapurines, such as 7-deazaadenine or 7-deazaguanine) or both (e.g.,
35 7-deaza, 8-azapurines). By derivatives of such bases or analogues are meant those bases wherein ring substituent are either incorporated, removed, or modified by

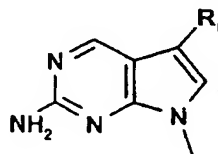
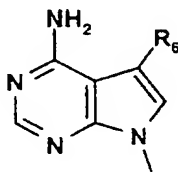
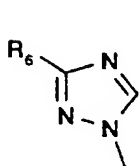
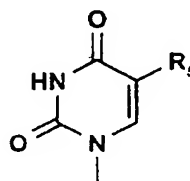
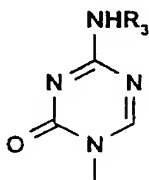
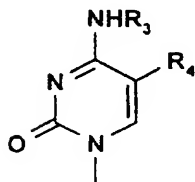
conventional substituents known in the art, e.g. halogen, hydroxyl, amino, C₁₋₆ alkyl. Such purine or pyrimidine bases, analogs and derivatives are well known to those of skill in the art.

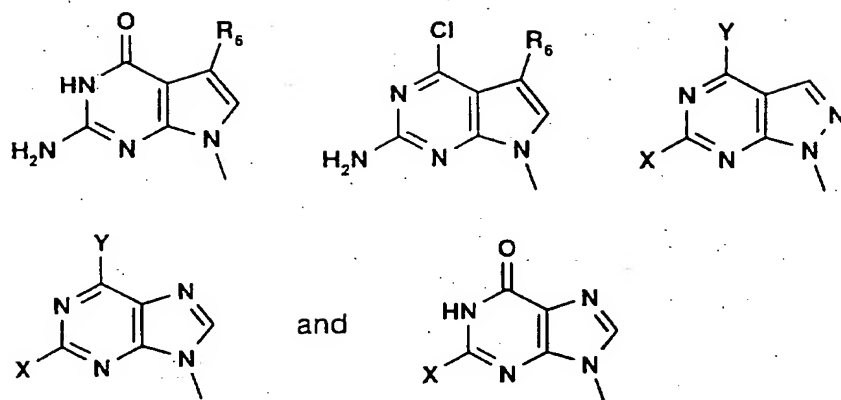
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R₁ is a hydroxyl protecting group. Suitable protecting groups include those described in detail in Protective Groups in Organic Synthesis, Green, John, J. Wiley and Sons, New York (1981). Preferred hydroxyl protecting

- 10 groups include ester forming groups such as C₁₋₆ acyl i.e. formyl, acetyl, substituted acetyl, propionyl, butanoyl, pivalamido, 2-chloroacetyl; aryl substituted C₁₋₆ acyl i.e. benzoyl, substituted benzoyl; C₁₋₆ alkoxy carbonyl i.e. methoxycarbonyl; aryloxy carbonyl i.e. phenoxycarbonyl.
- 15 Other preferred hydroxyl protecting groups include ether forming groups such as C₁₋₆ alkyl i.e. methyl, t-butyl; aryl C₁₋₆ alkyl i.e. benzyl, diphenylmethyl any of which is optionally substituted i.e. with halogen. Particularly preferred hydroxyl protecting groups are t-butoxycarbonyl,
- 20 benzoyl and benzyl each optionally substituted with halogen. In a more particularly preferred embodiment the R₁ hydroxyl protecting group is benzyl.

- 25 In a preferred embodiment, R₂ is selected from the group consisting of

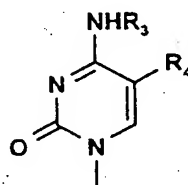




wherein

- 5 R_1 is selected from the group consisting of hydrogen, C_{1-6} alkyl and C_{1-6} acyl groups;
- R_2 and R_3 are independently selected from the group consisting of hydrogen, C_{1-6} alkyl, bromine, chlorine, fluorine, and iodine;
- 10 R_4 is selected from the group of hydrogen, halogen, cyano, carboxy, C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} alkoxy, C_{1-6} acyl, C_{1-6} acyloxy, carbamoyl, and thiocarbamoyl; and
- X and Y are independently selected from the group of hydrogen, bromine, chlorine, fluorine, iodine, amino,
- 15 and hydroxyl groups.

In a particularly preferred embodiment R_1 is



20

wherein R_2 and R_4 are as previously defined.

In a particularly preferred embodiment R_1 is cytosine or an analogue or derivative thereof. Most preferably R_1 is cytosine, N-acetylcytosine or N-acetyl-5-fluorocytosine.

- 5 In preferred embodiments R_2 is H. In another preferred embodiment R_2 is C_{1-4} acyl such as acetyl.

- In preferred embodiments R_3 and R_4 are independently selected from hydrogen, C_{1-4} alkyl such as methyl or ethyl
 10 and halogen such as F, Cl, I or Br. In particularly preferred embodiments R_3 and R_4 are hydrogen. In another particularly preferred embodiment R_3 and R_4 are F.

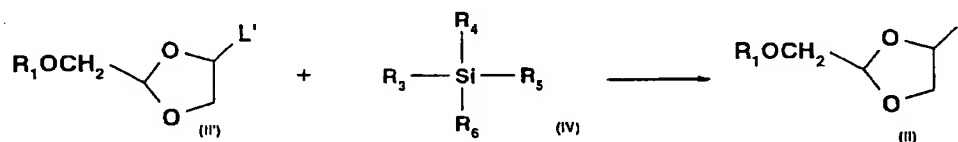
- In preferred embodiments R_5 is selected from hydrogen,
 15 halogen, carboxy and C_{1-4} alkyl. In particularly preferred embodiments R_5 is H, F or Cl and most preferably H.

- In preferred embodiments X and Y are independently selected from the group of H, F or Cl. In a particularly
 20 preferred embodiment X and Y are hydrogen.

L is selected from the group consisting of fluoro, bromo, chloro and iodo.

- 25 In a particularly preferred embodiment L is an iodo group. In this instance, leaving group (L) may be prepared by displacement of another leaving group (L') i.e. acetoxy with Lewis acids containing an iodo moiety. Preferably such Lewis acids have the formula (IV):

30



wherein R_3 , R_4 and R_5 are independently selected from the group consisting of hydrogen; C_{1-20} alkyl (e.g. methyl,

ethyl, ethyl, t-butyl), optionally substituted by halogens (F, Cl, Br, I), C₆₋₂₀ alkoxy (e.g., methoxy) or C₆₋₂₀ aryloxy (e.g., phenoxy); C₇₋₂₀ aralkyl (e.g., benzyl), optionally substituted by halogen, C₁₋₂₀ alkyl or C₁₋₂₀ alkoxy (e.g., p-methoxybenzyl); C₆₋₂₀ aryl (e.g., phenyl), optionally substituted by halogens, C₁₋₂₀ alkyl or C₁₋₂₀ alkoxy; trialkylsilyl; fluoro; bromo; chloro and iodo; and R₁ is selected from the group consisting of halogen (F, Cl, Br, I) preferably I (iodo);

10

L' is a leaving group capable of being displaced by an iodo leaving group using a Lewis acid of formula (IV). Suitable leaving groups L' include acyloxy; alkoxy; alkoxy carbonyl; amido; azido; isocyanato; substituted or unsubstituted, saturated or unsaturated thiolates; substituted or unsubstituted, saturated or unsaturated seleno, seleninyl or selenonyl compounds; -OR wherein R is a substituted or unsubstituted, saturated or unsaturated alkyl group; a substituted or unsubstituted, aliphatic or aromatic acyl group; a substituted or unsubstituted, saturated or unsaturated alkoxy or aryloxy carbonyl group; substituted or unsubstituted sulphonyl imidazolidine; substituted or unsubstituted, aliphatic or aromatic amino carbonyl group; substituted or unsubstituted alkyl imidate group; substituted or unsubstituted, saturated or unsaturated phosphonate; and substituted or unsubstituted, aliphatic or aromatic sulphinyl or sulphonyl group. In a preferred embodiment L' is acetoxy.

30

In a preferred embodiment, the present invention provides a stereoselective process for producing β -nucleoside analogues of formula (III), and salt or ester thereof, by glycosylation of the purine or pyrimidine base or analogue or derivative thereof, with an intermediate of formula (II) as defined previously under low temperature conditions. Preferably, the glycosylation reaction takes

place at temperatures below -10°C i.e. about -10 to -100°C and more preferably below -20°C . In a most preferred embodiment the glycosylation reaction occurs between about -20 to -78°C .

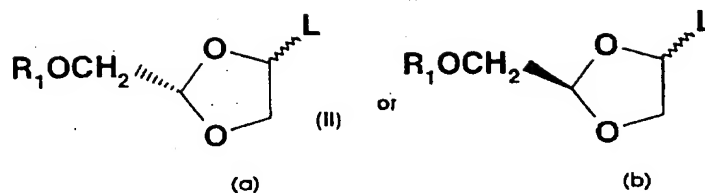
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The intermediate of formula II is reacted with a silylated purine or pyrimidine base, conveniently in a suitable organic solvent such as a hydrocarbon, for example, toluene, a halogenated hydrocarbon such as dichloromethane (DCM), a nitrile, such as acetonitrile, an amide such as dimethylformamide, an ester, such as ethyl acetate, an ether such as tetrahydrofuran, or a mixture thereof, at low temperatures, such as -40°C to -78°C . Silylated purine or pyrimidine bases or analogues and derivatives thereof may be prepared as described in WO92/20669, the teaching of which is incorporated herein by reference. Such silylating agents are 1,1,1,3,3,3-hexamethyldisilazane, trimethylsilyl triflate, t-butyltrimethylsilyl triflate or trimethylsilyl chloride, with acid or base catalyst, as appropriate. The preferred silylating agent is 1,1,1,3,3,3-hexamethyldisilazane.

To form the compound of formula (I), appropriate deprotecting conditions include methanolic or ethanolic ammonia or a base such as potassium carbonate in an appropriate solvent such as methanol or tetrahydrofuran for N-4 deacetylation.

Transfer deacetylation hydrogenolysis with a hydrogen donor such as cyclohexene or ammonium formate in the presence of a catalyst such as palladium oxide over charcoal are appropriate for the removal of the 5'-aryl group.

It will be appreciated that the intermediate of formula (II) is constituted by intermediates IIa and IIb:



It will be further appreciated that, if the glycosylation
 5 step is carried out using equimolar amounts of
 intermediates IIa and IIb, a racemic mixture of β -
 nucleosides of formula I is obtained.

It will be apparent to those of skill in the art that
 10 separation of the resulting diastereomic mixture, for
 example after the coupling reaction between compounds of
 formula II and a silylated base, can be achieved by
 chromatography on silica gel or crystallization in an
 appropriate solvent (see, for example: J. Jacques et al.
 15 *Enantiomers, Racemates and Resolutions*, pp 251-369, John
 Wiley and Sons, New York 1981).

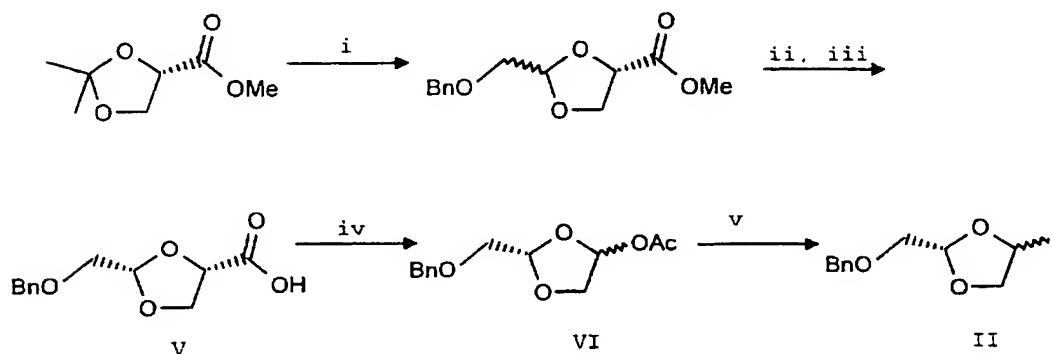
However, it is preferred that glycosylation is effected
 using an optically pure compound of either formula IIa or
 20 IIb, thereby producing the desired nucleoside analog in
 high optical purity.

The compounds of formula IIa or IIb exist as mixture of
 two diastereomers epimeric at the C-4 centre. We have now
 25 found that a single diastereomer, as well as any mixture
 of the diastereomers comprising the compounds of formula
 IIa, react with silylated bases to produce β -L nucleosides
 in high optical purity. The base at C-4 having the *cis*-
 stereochemistry relative to the hydroxymethyl moiety at C-
 30 2. The rate of the reaction of the two diastereomers of
 formula IIa with silylated bases may however, be
 different. Similar findings exist for the intermediates
 of formula IIb for the synthesis of β -D nucleosides.

In a preferred embodiment, the present invention provides a step for producing anomeric iodides of formula II by reacting known anomeric 2S-benzoyloxymethyl-1,3-dioxolane-4S and -4R acetoxy derivatives of formula (V) with iodotrimethylsilane or diiodosilane at low temperatures (-78°C) prior to glycosylation with silylated pyrimidine or purine base or analogue or derivative thereof (Scheme 1).

10

Scheme 1



15 Reagents and conditions:

- i) $\text{BnO}-\text{CH}_2-\text{CH}=\text{O}$ / Toluene TSHO/80%/2.7:1.0 cis/trans;
 ii) MeOH/LiOH;
 iii) Column separation;
 iv) $\text{Pb}(\text{OAc})_4/\text{MeCN}/\text{Py}/2\text{h}/\text{RT}/80\%$; and
 20 v) TMSI or SiH_2I_2 / CH_2Cl_2 / -78°C .

Suitable methods for producing the anomeric acetoxy intermediate (VI) will be readily apparent to those skilled in the art and include oxidative degradation of benzyloxymethylacetals derived from L-ascorbic acid (Belleau et al. *Tetrahedron Lett.* 1992, 33, 6949-6952) or

D-mannitol (Evans et al. *Tetrahedron Asymmetry* 1993, 4, 2319-2322).

We have also found that the known 2S-benzyloxymethyl-1,3-dioxolane-4S-carboxylic acid (V) can be generated in preference to its 2S,4R isomer by reacting commercially available 2,2-dimethyl-1,3-dioxolane-4S-carboxylic acid with a protected derivative of hydroxyacetaldehyde, such as benzyloxyacetaldehyde, under acidic conditions.

10

In the diastereoselective process of this invention, there is also provided the following intermediates:

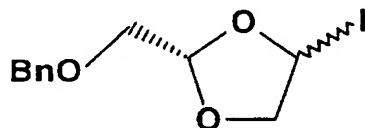
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- 2S-Benzyloxymethyl-4R-iodo-1,3 dioxolane and 2S-Benzyloxymethyl-4S-iodo-1,3 dioxolane;
- β -L-5'-Benzyl-2'-deoxy-3'-oxa-N-4-acetylcytidine;
- β -L-5'-Benzyloxy-2'-deoxy-3'-oxacytidine;
- β -L-5'-Benzyl-2'-deoxy-3'-oxa-5-fluoro-N4-acetylcytidine; and
- β -L-5'-Benzyl-2'-deoxy-3'-oxa-5-fluorocytidine.

20

Example 1a: 2S-Benzyloxymethyl-4R-iodo-1,3 dioxolane
and 2S-Benzyloxymethyl-4S-iodo-1,3
dioxolane (compound #1)

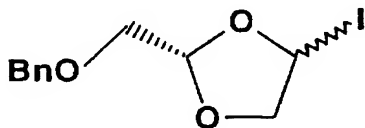
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A mixture consisting of 2S-benzyloxymethyl-4S acetoxymethyl-1,3
dioxolane and 2S-benzyloxymethyl-4R-acetoxymethyl-1,3 dioxolane
10 in 1:2 ratio (6g; 23.8 mmol) was dried by azeotropic
distillation with toluene *in vacuo*. After removal of
toluene, the residual oil was dissolved in dry
dichloromethane (60 ml) and iodotrimethylsilane (3.55 ml;
1.05 eq) was added at -78°C, under vigorous stirring. The
15 dry-ice/acetone bath was removed after addition and the
mixture was allowed to warm up to room temperature (15
min.). The ¹H NMR indicated the formation of 2S-
benzyloxymethyl-4R-iodo-1,3-dioxolane and
2S-benzyloxymethyl-4S-iodo-1,3 dioxolane.

20 ¹H NMR (300 MHz, CDCl₃) δ 3.65-4.25 (2H,m); 4.50-4.75
(4H,m) 5.40-5.55 (1H, overlapping triplets); 6.60-6.85
(1H, d of d); 7.20-7.32 (5H,m).

Example 1b: 2S-Benzyloxymethyl-4R-iodo-1,3 dioxolane
25 and 2S-Benzyloxymethyl-4S-iodo-1,3
dioxolane (compound #1)

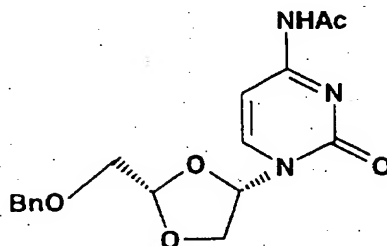


30 A mixture consisting of 2S-benzyloxymethyl-4S acetoxymethyl-1,3
dioxolane and 2S-benzyloxymethyl-4R-acetoxymethyl-1,3 dioxolane

in 1:2 ratio (6g; 23.8 mmol) was dried by azeotropic distillation with toluene in vacuo. After removal of toluene, the residual oil was dissolved in dry dichloromethane (60 ml) and diiodosilane (2.4 ml; 1.05 eq) was added at -78°C, under vigorous stirring. The dry-ice/acetone bath was removed after addition and the mixture was allowed to warm up to room temperature (15 min.). The ¹H NMR indicated the formation of 2S-benzyloxymethyl-4R-iodo-1,3-dioxolane and 2S-benzyloxymethyl-4S-iodo-1,3 dioxolane.

¹H NMR (300 MHz, CDCl₃) δ 3.65-4.25 (2H,m); 4.50-4.75 (4H,m) 5.40-5.55 (1H, overlapping triplets); 6.60-6.85 (1H, d of d); 7.20-7.32 (5H,m).

Example 2: β-L-5'-Benzyl-2'-deoxy-3'-oxa-N-4-acetyl-cytidine (compound #2)

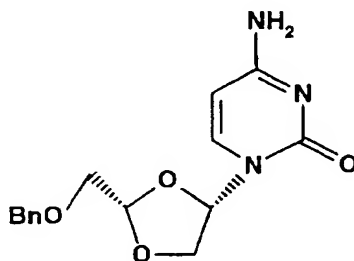


The previously prepared iodo intermediate (example 1) in dichloromethane, was cooled down to -78° C. Persilylated N-acetyl cytosine (1.1 eq) formed by reflux in 1,1,1,3,3,3-hexamethyl disilazane (HMDS) and ammonium sulphate followed by evaporation of HMDS was dissolved in 30 ml of dichloromethane and was added to the iodo intermediate. The reaction mixture was maintained at -78°C for 1.5 hours then poured onto aqueous sodium bicarbonate and extracted with dichloromethane (2 x 25 ml). The organic phase was dried over sodium sulphate, the solid was removed by filtration and the solvent was evaporated in vacuo to produce 8.1 g of a crude mixture. Based on

¹H NMR analysis, the β-L-5'-benzyl-2'-deoxy-3'-oxacytidine and its α-L isomer were formed in a ratio of 5:1 respectively. This crude mixture was separated by chromatography on silica-gel (5% MeOH in EtOAc) to generate the pure β-L (cis) isomer (4.48 g). Alternatively, recrystallization of the mixture from ethanol produces 4.92 g of pure β isomer and 3.18 g of a mixture of β and α-isomers in a ratio of 1:1.

¹H NMR (300 MHz, CDCl₃) δ 2.20 (3H, s, Ac); 3.87 (2H, m, H-5'), 4.25 (2H, m, H-2'); 4.65 (2H, dd, OCH₂Ph); 5.18 (1H, t, H-4'); 6.23 (1H, m, H-1'); 7.12 (1H, d, H-5); 7.30-7.50 (5H, m, Ph); 8.45 (2H, m, NH+H-6).

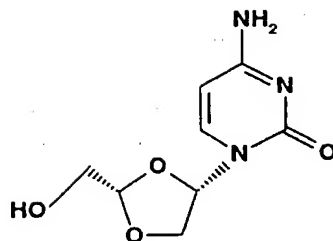
Example 3: β-L-5'-Benzyloxy-2'-deoxy-3'-oxacytidine
(compound #3)



The protected β-L isomer (4.4 g) of example 2 was suspended in saturated methanolic ammonia (250 ml) and stirred at room temperature for 18 hours in a closed vessel. The solvents were then removed in vacuo to afford the deacetylated nucleoside in pure form.

¹H NMR (300 MHz, CDCl₃) δ 3.85 (2H, m, H-5'); 4.20 (2H, m, H-2'); 4.65 (2H, dd, OCH₂Ph); 5.18 (1H, t, H-4'); 5.43 (1H, d, H-5); 5.50-5.90 (2H, br. s, NH₂); 6.28 (1H, m, H-1'); 7.35-7.45 (5H, m, Ph); 7.95 (1H, d, H-6).

Example 4: β-L-2'-deoxy-3'-oxacytidine (compound #4)

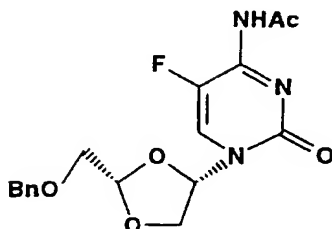


β -L-5'-Benzyl-2'-deoxy-3'-oxacytidine from the previous example, was dissolved in EtOH (200 ml) followed by
 5 addition of cyclohexene (6 ml) and palladium oxide (0.8 g). The reaction mixture was refluxed for 7 hours then it was cooled and filtered to remove solids. The solvents were removed from the filtrate by vacuum distillation. The crude product was purified by flash chromatography on
 10 silica-gel (5% MeOH in EtOAc) to yield a white solid (2.33 g; 86% overall yield, $\alpha_D^{25} = -46.7^\circ$ (c = 0.285; MeOH) m.p. = 192 - 194°C.

^1H NMR (300 MHz, DMSO- d_6) δ 3.63 (2H, dd, H-5'); 4.06 (2H, m, H-2'); 4.92 (1H, t, H-4'); 5.14 (1H, t, OH); 5.70
 15 (1H, d, H-5); 6.16 (2H, dd, H-1'); 7.11 - 7.20 (2H, brs, NH_2); 7.80 (1H, d, H-6) ^{13}C NMR (75 MHz, DMSO- d_6) δ 59.5 (C-2'); 70.72 (C-5'); 81.34 (C-4'); 93.49 (C-1'); 104.49 (C-5); 140.35 (C-4); 156.12 (C-6); 165.43 (C-2).

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Example 5: β -L-5'-Benzyl-2'-deoxy-3'-oxa-5-fluoro-N4-acetyl-cytidine (compound #5)



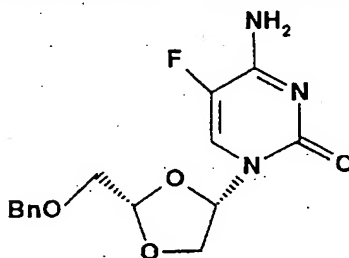
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The previously prepared iodo derivatives (example 1) in dichloromethane, was cooled down to -78°C . Persilylated N-acetyl-5-fluorocytosine (1.05 eq) formed by reflux in 1,1,1,3,3,3-hexamethyldisilazane (HMDS) and ammonium sulphate followed by evaporation of HMDS was dissolved in 20 ml of dichloromethane (DCM) and was added to the iodo intermediate. The reaction mixture was maintained at -78°C for 1.5 hours then poured onto aqueous sodium bicarbonate and extracted with dichloromethane (2 x 25 ml). The organic phase was dried over sodium sulphate, the solid was removed by filtration and the solvent was evaporated in vacuo to produce 8.1 g of a crude mixture. Based on ^1H NMR analysis, the β -L-5'-benzyl-2'-deoxy-3'-oxa-5-fluoro-N4-acetyl-cytidine and its α -L isomer were formed in a ratio of 5:1 respectively. This crude mixture was separated by chromatography on silica-gel (5% MeOH in EtOAc) to generate the pure β -L (*cis*) isomer (4.48 g). Alternatively, recrystallization of the mixture from ethanol produces 4.92 g of pure β isomer and 3.18 g of a mixture of β and α -isomers in a ratio of 1:1.

^1H NMR (300 MHz, CDCl_3) δ 2.20 (3H, s, Ac); 3.87 (2H, m, H-5'), 4.25 (2H, m, H-2'); 4.65 (2H, dd, OCH_2Ph); 5.18 (1H, t, H-4'); 6.23 (1H, m, H-1'); 7.12 (1H, d, H-5); 7.30-7.50 (5H, m, Ph); 8.45 (2H, m, NH+H-6).

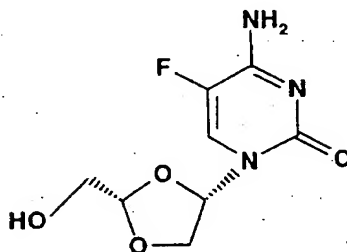
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Example 6: β -L-5'-Benzyl-2'-deoxy-3'-oxa-5-fluorocytidine (compound #6):



5 The crude mixture from previous step (example 5) was suspended in methanolic ammonia (100 ml) and stirred for 18 hours at room temperature in a closed reaction vessel. The solvents were removed in vacuo to afford the deacetylated mixture which was separated by flash
10 chromatography on silica gel (2% to 3% MeOH in EtOAc) to yield 1.21 g pure β isomer (yield 45% with respect to this isomer).

15 Example 7: β -L-2'-deoxy-3'-oxa-5-fluorocytidine (compound #7)



The deacetylated pure β -L isomer (900 mg; 2.8 mmol)
20 prepared as described in example 6 was dissolved in EtOH (40 ml) followed by addition of cyclohexene (3 ml) and palladium oxide catalyst (180 mg). The reaction was refluxed for 24 hours and the catalyst was removed by
filtration. The solvents were removed from the filtrate
25 by vacuum distillation. The crude product was purified by

flash chromatography on silica-gel (5% to 7% MeOH in EtOAc) to yield a white solid (530 mg ; 82% yield). (α_D^{22}) = -44.18° (c = 0.98; MeOH).

¹H NMR (300 MHz, DMSO-d₆); δ 3.62-3.71 (2H,m,H-5'); 4.03-4.13 (2H;m,H-2'); 4.91 (1H,t,H-4'); 5.32 (1H,t,OH); 6.11 (1H;t,H-1'); 7.53-7.79 (2H,b,NH₂); 8.16 (1H;d,H-6); ¹³C NMR (75 MHz, DMSO-d₆); δ 59.34 (C-2'); 70.68 (C-5'); 80.78 (C-4'); 104.53-(C-1'); 124.90, 125.22 (C-4); 134.33, 136.73 (C-5); 153.04 (C-2); 156.96, 157.09 (C-6).

10

Example 8: Isomeric purity determination of β -L-2'-deoxy-3'-oxacytidine nucleoside analogues:

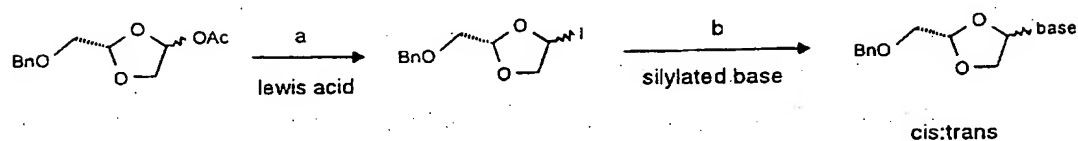
The determination of the isomeric purity (β -L versus α -L and β -L versus β -D isomers) was determined on a Waters HPLC system consisting of a 600 controller pump for solvent delivery, 486 uv detector, 412 WISP auto sampler and a 740 Waters integrator module. An analytical chiral reverse phase cyclobond I RSP column (Astec, 4.6 x 250 mm i.d.) was used and packed by the manufacturer with β -cyclodextrin derivatized with R'S-hydroxypropyl ether. The mobile phase consisted of acetonitrile (A) and water containing 0.05% triethylamine (B) with the pH adjusted to 7.05 by glacial acetic acid. The column was operated under isocratic conditions at 0° C using a mixture of 5% A and 95% B. Such conditions are modifications of those reported in DiMarco et al. (*J. Chromatography*, 1993, 645, 107-114). The flow rate was 0.22 ml/min and the pressure was maintained at 648 - 660 psi. Detection of nucleosides was monitored by uv absorption at 215 and 265 nm. Samples of β -D isomer and racemic compounds were prepared as reported (Belleau et al. *Tetrahedron Lett* 1992, 33, 6948-6952) and used for internal references and co-injection.

Under these conditions the isomeric purity of compound #4 produced according to example 4 was > 99% and that of compound #7 according to example 7, was > 96%.

- 5 The isomeric purity of dioxolane nucleosides having been prepared according to the general scheme 2, under varying conditions i.e. temperature and Lewis acid is represented in table 1 below. Those prepared at temperatures above -10°C exhibited reduced stereoselectivity.

10

Scheme 2



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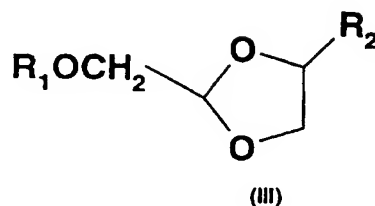
Table 1

Base	Lewis acid	Temperature(°c)		Cis : trans
5F-N(Ac)-cytosine	TMSI	a:-78	b:-78	8 : 1
5F-N(Ac)-cytosine	SiH ₂ I ₂	a:-78	b:-78	7 : 2
N(Ac)-cytosine	TMSI	a:-78	b:-78	5 : 1

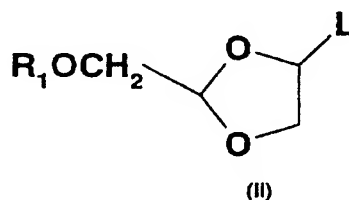
note : all reactions in DCM solvent and bases silylated with HMDS.

WE CLAIM:

1. A process for producing a β -nucleoside analogue compound of formula (III):



and salts thereof, wherein R_1 is a hydroxyl protecting group; and R_2 is a purine or pyrimidine base or an analogue or derivative thereof, the process comprising glycosylating said purine or pyrimidine base at a temperature below about -10°C , with an intermediate of formula (II):



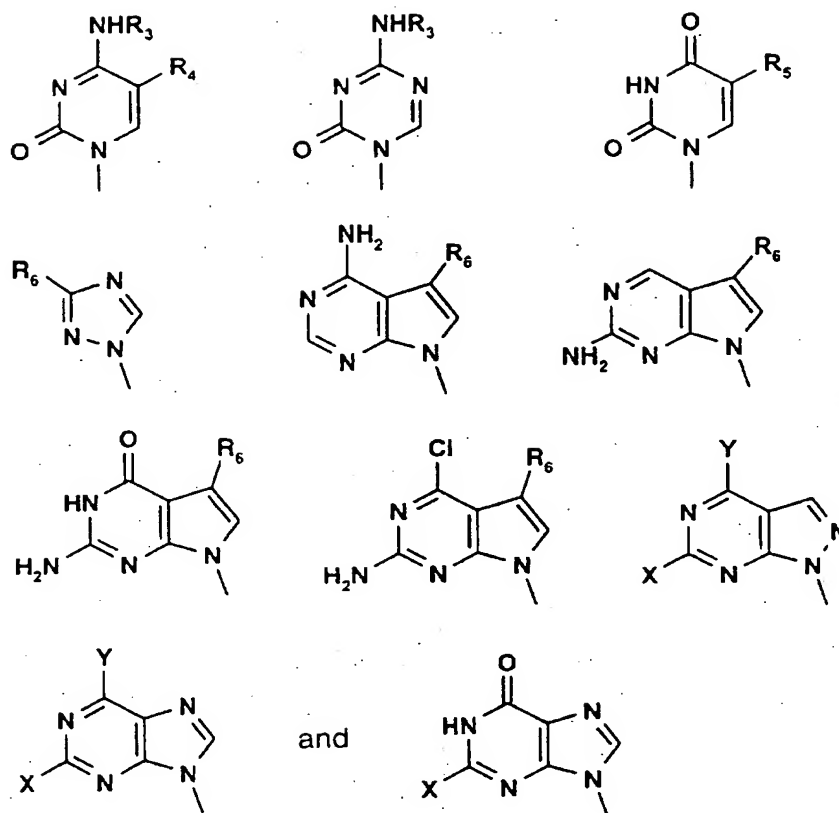
15 wherein L is halogen.

2. The process according to claim 1, wherein L is iodo.

- 20 3. The process according to claim 2, wherein R_1 is benzyl.

4. The process according to claim 1, wherein R_2 is selected from the group consisting of

25



5

wherein

R_1 is selected from the group consisting of hydrogen, C_{1-6} alkyl and C_{1-6} acyl groups;

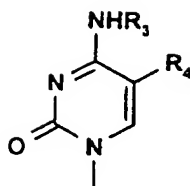
10 R_4 and R_6 are independently selected from the group consisting of hydrogen, C_{1-6} alkyl, bromine, chlorine, fluorine, and iodine;

R_5 is selected from the group of hydrogen, halogen, cyano, carboxy, C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} acyl, C_{1-6} acyloxy, carbamoyl, and thiocarbamoyl; and

15 X and Y are independently selected from the group of hydrogen, bromine, chlorine, fluorine, iodine, amino, and hydroxyl groups.

5. The process according to claim 1, wherein R_1 is

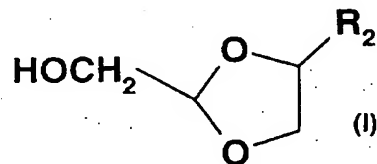
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wherein

- 5 R_3 is selected from the group consisting of hydrogen, C_{1-6} alkyl and C_{1-6} acyl groups; and
- R_4 is selected from the group consisting of hydrogen, C_{1-6} alkyl, bromine, chlorine, fluorine, and iodine.
- 10 6. The process according to claim 5, wherein R_3 is H or acetyl and R_4 is H or F.
7. The process according to claim 1, wherein the glycosylation reaction occurs at a temperature below about -15°C .
- 15 8. The process according to claim 1, wherein the glycosylation reaction occurs at a temperature below about -20°C .
- 20 9. The process according to claim 1, wherein the glycosylation reaction occurs at a temperature below about -50°C .
- 25 10. The process according to claim 1, wherein the glycosylation reaction occurs at about -78°C .
11. The process according to any one of claims 7 to 10 wherein L is iodo.
- 30 12. The process according to claim 11 wherein R_1 is benzyl.

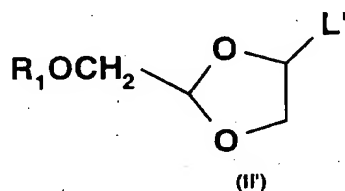
13. The process according to claim 1, wherein the compound of formula (III) is subsequently deprotected to give a compound of formula (I)



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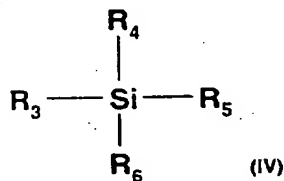
wherein R_2 is a purine or pyrimidine base or an analogue or derivative thereof.

- 10 14. The process according to claim 1, wherein the intermediate of formula (II) is prepared by reacting an intermediate of formula (II')



15

wherein L' is a leaving group; with a Lewis acid of formula (IV)



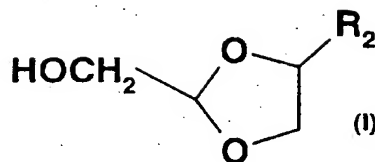
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wherein

R_1 , R_4 and R_5 are independently selected from the group consisting of hydrogen; C_{1-20} alkyl (e.g. methyl, ethyl, ethyl, t-butyl), optionally substituted by halogens (F, Cl, Br, I), C_{6-20} alkoxy (e.g., methoxy) or C_{6-20} aryloxy (e.g., phenoxy); C_{7-20} aralkyl (e.g., benzyl), optionally substituted by

25

- halogen, C₁₋₂₀ alkyl or C₁₋₂₀ alkoxy (e.g., p-methoxybenzyl); C₆₋₂₀ aryl (e.g., phenyl), optionally substituted by halogens, C₁₋₂₀ alkyl or C₁₋₂₀ alkoxy; trialkylsilyl; fluoro; bromo; chloro and iodo; and
- 5 R₄ is selected from the group consisting of halogen (F, Cl, Br, I); C₁₋₂₀ sulphonate esters, optionally substituted by halogens (e.g., trifluoromethane sulphonate); C₁₋₂₀ alkyl esters, optionally
- 10 substituted by halogen (e.g., trifluoroacetate); polyvalent halides (e.g., triiodide); trisubstituted silyl groups of the general formula (R₁)(R₂)(R₃)Si (wherein R₁, R₂, R₃ are as defined above); saturated or unsaturated selenenyl C₆₋₂₀
- 15 aryl; substituted or unsubstituted C₆₋₂₀ arylsulphenyl; substituted or unsubstituted C₁₋₂₀ alkoxyalkyl; and trialkylsiloxy.
15. The process according to claim 14, wherein the Lewis
- 20 acid is selected from TMSI and SiH₂I₂.
16. The process according to claim 15, wherein the Lewis acid is TMSI.
- 25 17. The process according to claim 14, wherein L is iodo.
18. The process according to claim 17, wherein R₁ is benzyl.
- 30 19. The process according to any one of claims 14 to 18, wherein the compound of formula (III) is subsequently deprotected to give a compound of formula (I)



wherein R_1 is a purine or pyrimidine base or an analogue or derivative thereof.

- 5
20. The process according to claim 19, wherein the glycosylation reaction occurs at a temperature below about -15°C .
- 10 21. The process according to claim 19, wherein the glycosylation reaction occurs at a temperature below about -20°C .
22. The process according to claim 19, wherein the glycosylation reaction occurs at a temperature below about -50°C .
- 15
23. The process according to claim 19, wherein the glycosylation reaction occurs at about -78°C .
- 20

INTERNATIONAL SEARCH REPORT

national Application No

PCT/CA 96/00845

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D411/04 C07H19/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07H C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP 0 577 304 A (ELI LILLY AND COMPANY) 5 January 1994 see the whole document ---	1
Y	WO 92 14729 A (EMORY UNIVERSITY) 3 September 1992 see the whole document ---	1
Y	TETRAHEDRON LETTERS, vol. 29, no. 11, 1988, OXFORD GB, pages 1239-1242, XP002025409 V.FARINA ET AL.: "A New Synthesis of 2',3'-Dideoxynucleosides for Aids Chemotherapy." cited in the application see the whole document --- -/-	1

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

17 February 1997

Date of mailing of the international search report

20 -03- 1997

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Authorized officer

Scott, J

INTERNATIONAL SEARCH REPORT

International Application No

PCT/CA 96/00845

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>TETRAHEDRON LETTERS, vol. 33, no. 46, 1992, OXFORD GB, pages 6949-6952, XP002025410 B.R.BELLEAU ET AL.: "Oxidative Degradation of L-Ascorbic Acid Acetals to 2',3'-Dideoxy-3'-Oxaribofuranosides. Synthesis of Enantiomerically Pure 2',3'-Dideoxy-3'-Oxacytidine Stereoisomers as Potential Antiviral Agents." cited in the application see the whole document -----</p>	1
Y	<p>JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, vol. 113, 1991, DC US, pages 9377-9379, XP002025411 W-B.CHOI ET AL.: "In Situ Complexation Directs the Stereochemistry of N-Glycosylation in the Synthesis of Oxathiolanyl and Dioxolanyl Nucleoside Analogues." cited in the application see the whole document -----</p>	1

INTERNATIONAL SEARCH REPORT

Information on patent family members

national Application No

PCT/CA 96/00845

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-577304	05-01-94	AU-B- 659008	04-05-95
		AU-A- 4135393	23-12-93
		AU-A- 4135593	23-12-93
		BR-A- 9302431	11-01-94
		BR-A- 9302434	16-02-94
		CA-A- 2098874	23-12-93
		CA-A- 2098881	23-12-93
		CN-A- 1086519	11-05-94
		CZ-A- 9301234	16-02-94
		EP-A- 0577303	05-01-94
		HU-A- 64358	28-12-93
		HU-A- 64359	28-12-93
		JP-A- 6157570	03-06-94
		JP-A- 6073086	15-03-94
		NO-A- 932286	23-12-93
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		NZ-A- 247936	26-05-95
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		PL-A- 299413	07-03-94
		PL-A- 299415	07-03-94
WO-A-9214729	03-09-92	US-A- 5210085	11-05-93
		US-A- 5276151	04-01-94
		AU-A- 1437292	15-09-92
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		AU-A- 1561792	15-09-92
		AU-A- 3794395	14-03-96
		BG-A- 98062	25-04-94
		BR-A- 9205661	24-05-94
		CA-A- 2104399	23-08-92
		CN-A- 1065065	07-10-92
		CN-A- 1127301	24-07-96
		EP-A- 0575482	29-12-93
		HU-A- 65548	28-06-94
		HU-A- 9500510	28-11-95
		JP-T- 6508605	29-09-94
		NZ-A- 241625	26-03-96
		NZ-A- 250842	26-03-96
		PL-B- 169842	30-09-96
		WO-A- 9214743	03-09-92

Information on patent family members

PCT/CA 96/00845

Form PCT/ISA/210 (patent family annex) (July 1992)

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